Results: In SLE patients respect to HC, plasma IL-1 β levels were unmodified whereas IL-6 was higher, resulting significantly increased in SLE-S. Monocytes isolated from SLE patients released lower quantities of IL-1 β after stimulation with BzATP, whereas the release of both IL-6 and TNF- α was significantly augmented in SLE-NS respect to both HC and SLE-S subjects after all types of stimulation. RT-PCR showed reduced P2X7R and augmented NLRP3 mRNA expression in SLE patients. Accordingly, P2X7R activity was significantly reduced in all SLE patients and did not appear to be influenced by a chloroquine pre-treatment.

Conclusion: In SLE patients, compared to HC subjects, we found reduced P2X7R mRNA expression, increased NLRP3 mRNA, as a possible compensating mechanism, and correspondingly, significantly lower BzATP-induced intracellular Ca²⁺ increase, without an apparent influence by chloroquine, one of the drugs most diffusely used for SLE treatment. The *in vitro* IL-1 β release was reduced, whereas plasma IL-1 β was unaltered, indicating an alternative source, other than monocytes, of this cytokine. Conversely, IL-6 and TNF- α levels were increased *in vitro*, and IL-6 was present in plasma at higher levels. The possible consequences of reduced P2X7R, mainly on cytokines network deregulation and lymphocyte proliferation, will be further investigated as well as the role of IL-6 and TNF- α as possible therapeutic targets.

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THU0017 IN VITRO MECHANISTIC STUDIES DEMONSTRATE FILGOTINIB ACTIVITY THAT HAS POTENTIAL IMPLICATIONS FOR DIFFERENTIATION AMONG JAK INHIBITORS

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Background: Inhibition of the Janus kinase-signal transducers and activators of transcription (JAK-STAT) pathway has demonstrated efficacy in immune-mediated diseases and has been identified as a therapeutic target for the treatment of rheumatoid arthritis (RA). Differences in JAK inhibitor specificity for JAK1, JAK2, JAK3, and TYK2 may influence their safety profiles, but the mechanism is not known. Selective JAK1 inhibition by filgotinib (FIL) may modulate a subset of proinflammatory cytokines associated with RA pathogenesis and improve the risk-benefit profile by minimizing other non-JAK1-related adverse events. JAK2 inhibition is associated with cytopenias, while JAK3 inhibition has been associated with increased risk for opportunistic infections (eg, tuberculosis and herpes zoster) and chronic low-grade inflammation. In clinical trials, FIL did not negatively impact hemoglobin, LDL/HDL ratios, or natural killer (NK) cell counts.¹⁻³

Objectives: To compare the *in vitro* profile of JAK inhibitors with different JAK selectivity profiles, for effects on erythroid progenitor cell expansion, NK cell proliferation, and liver X receptor (LXR) agonist-induced cholesterol ester transfer protein (CETP) expression, an enzyme responsible for the conversion of HDL to LDL.

Methods: JAK inhibitors (FIL, FIL metabolite [GS-829845], baricitinib [BARI], tofacitinib [TOFA], and upadacitinib [UPA]) were evaluated *in vitro* in human cell-based assays: growth of erythroid progenitors from human cord blood CD34⁺ cells using a HemaTox™ liquid expansion assay, IL-15-induced NK cell proliferation, and LXR agonist-induced CETP expression in the hepatic cell line (HepG2). Using IC₅₀s generated from these

assays and the reported human plasma concentrations of the JAK inhibitors from clinical studies,⁴⁻⁶ we calculated the target coverage for each compound at clinically relevant doses. The activity of FIL in humans was based on a PK-PD modeling algorithm⁷ of FIL + GS-829845.

Results: *In vitro* assay results are described in the table. Based on these results, human exposure data, and modeled PK-PD relationships, FIL 100 mg and FIL 200 mg result in lower calculated cellular inhibition than the other JAK inhibitors at clinical exposures. Notably, FIL 100 mg and FIL 200 mg, but not the other inhibitors, are calculated to reduce CETP expression by 17% and 27%, respectively, while BARI, TOFA, and UPA are not expected to alter CETP levels.

Abstract THU0017 Table 1. IC₅₀ ± SD in *in vitro* assays (nM, unless otherwise noted).

Assay	FIL	GS-829845	BARI	TOFA	UPA
Early erythroid progenitors	1960 ±137	19300 ±1730	38.6±2.9	210 ±15.2	42 ±2.9
Mature erythroid progenitors	1140 ±112	10600 ±1270	25.7±2.9	110 ±10	24.5 ±2.75
NK cell proliferation	314.8 ±53	9697 ±8100	6.6±1.9	12.2 ±2.1	4.1 ±1.7
Inhibition of LXR agonist-induced CETP expression	15.3 ±7.1 μ M	19.4 ±4.2 μ M	>1 μ M weak induction ^a	No effect	No effect

^a Weak stimulation of LXR agonist-induced CETP expression.

Conclusion: JAK1 selectivity of FIL and GS-829845 resulted in less inhibition of erythroid progenitor expansion and NK cell proliferation compared with BARI, TOFA, and UPA. FIL also reduced LXR agonist-induced CETP expression, while the other inhibitors did not alter these levels. These results provide a potential mechanistic link to the observed reduction of CETP concentration and activity following FIL treatment, and the observed reduction in LDL:HDL in RA patients.⁸

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THU0018 ANTI-GALECTIN-9 ANTIBODY AS A NOVEL TREATMENT OPTION IN RHEUMATOID ARTHRITIS TARGETING PATHOGENIC FIBROBLAST-LIKE SYNOVIOCYTES

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Background: Fibroblasts-like synovocytes (FLS) present in the stromal environment are key effector cells in the persistence of synovial inflammation and joint damage in rheumatoid arthritis (RA). A particular subset of FLS characterized as Thy-1⁺Podoplanin⁺CD34⁺ is significantly expanded in RA patients and has been described to be disease-associated¹. Currently, no treatment targeting the stromal environment in RA is available².

Objectives: To identify a novel treatment option targeting the stromal environment in RA to modify the disease-associated subset of fibroblasts.

Methods: An *in vitro* model was established with FLS derived from synovial fluid mononuclear cells (SFMC) from RA and osteoarthritis (OA) patients (n=6). FLS between passage 2-5 were used for further analyses. Untreated FLS in cultures were analyzed by flow cytometry for expression

of the surface proteins CD34, CD45, Thy-1 and Podoplanin. The potential effect of cell detachment solutions on the expression of the surface proteins was examined by applying either trypsin or lidocaine. To evaluate on the inflammation status of the FLS, MCP-1 levels in supernatants from FLS in mono-cultures stimulated with either antibodies targeting galectin-9 (Gal-9), an anti-Gal-9 antibody-matched isotype control, LPS or steroid were analyzed by ELISA and compared to culture medium alone. Cell viability were examined using an MTT assay. Data are expressed as mean (95% CI) and analysed by a paired t-test. P-values <0.05 were considered statistically significant.

Results: FLS derived from SFMC were characterized as CD34⁺CD45⁺ and the majority co-expressed Thy-1 and Podoplanin [80.5%, (66.3-94.7%)] confirming the pathogenic phenotype of these cells. This phenotype was not altered by using different methods to detach the cells from the cell-culture plates. FLS receiving anti-Gal-9 antibody treatment showed a significant decreased fold change in MCP-1 secretion (0.84, [0.70;0.98]) compared with unstimulated cells (p=0.036). Treatment with an isotype control did not result in a significant decrease in MCP-1 secretion. This tendency was specific to FLS derived from RA patients as FLS derived from OA patients showed no significant decrease in MCP-1 secretion upon anti-Gal-9 antibody treatment. FLS derived from both RA or OA patients showed a significant increased fold change in secretion of MCP-1 upon LPS stimulation and significantly decreased levels of MCP-1 upon steroid treatment, consistent with the pathogenic phenotype of these cells. None of the different stimulations resulted in morphological changes of the FLS examined by light microscopy. Further, no significant changes in cell viability were detected after anti-Gal-9 antibody treatment.

Conclusion: FLS cultures derived from RA patients at passage 2-5 consist mainly of disease-associated fibroblasts and secrete significantly lower amounts of MCP-1 when treated with an anti-Gal-9 antibody without affecting cell viability. Thus suggesting that Gal-9 neutralization may represent a novel treatment option targeting the stromal environment and inflammation in RA.

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THU0019 INHIBITION OF FUCOSYLATION IN ENDOTHELIAL CELLS REDUCES RHEUMATOID ARTHRITIS ANGIOGENESIS

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Background: Glycosylation has been reported to associate with tumor invasion and metastasis. Fucosylation is involved the biological functions of adhesion molecules and growth factor receptors. In regards to arthritis, we have previously reported that fucosylated proteins were expressed in rheumatoid arthritis (RA) synovial tissues. However, a direct role for fucosylated cytokines in RA has not been demonstrated.

Objectives: To confirm that fucosylated proteins are expressed in RA and are involved in RA angiogenesis.

Methods: Total glycans were determined in serum from normal (NL) subjects and RA patients using mass spectrometry. N-glycans from 10 RA or 10 healthy controls, and 10 pretreatment with tocilizumab (TCZ) or after treatment sera at 24 weeks were purified by glycoblotting using BlotGlycoH. To determine whether fucosylated proteins involved with RA inflammation, the correlation with disease activity score (DAS) 28 (ESR) was measured. 2-deoxy-D-galactose (2-dGal) is an analog of hexose that inhibits fucosylation. In order to confirm the role of fucosylation in RA angiogenesis, we did Matrigel assays *in vitro*. To block the expression of fucosylated proteins, human umbilical vein endothelial cells (HUVECs) were treated with 2-dGal (15 mM) for 5 days. After treatment with 2-dGal, HUVECs were plated on Matrigel and were incubated with phosphate buffered saline (PBS) or RA synovial fluids. In addition, chemotaxis assays were performed to determine the role of fucosylation in HUVEC migration. Finally, expression of proangiogenic cytokines such as

fractalkine/CX3CL1, CXCL16, interleukin (IL)-8/CXCL8, monocyte chemotactic protein 1 (MCP-1)/CCL2, epithelial neutrophil-activating protein 78 (ENA-78)/CXCL5 and vascular endothelial growth factor (VEGF) in 2-dGal treated HUVEC conditioned medium were measured by ELISA.

Results: Total glycans in RA serum were significantly higher than in NL serum [mean ± SEM; 477 ± 24 pmol/μl (n=10) and 339 ± 14 pmol/μl (n=10), p<0.05, respectively]. In addition, total glycans in RA serum were significantly decreased with tocilizumab treatment at 24 weeks. Total glycans in RA serum were also correlated with DAS28 (ESR). Percent of fucosylated proteins in total glycans were decreased with TCZ treatment at 24 weeks. 2-d Gal treated HUVEC tube formed towards RA synovial fluids (n=6 patients) were decreased compared with nontreated HUVEC tube formed (number of tube formed; 7 ± 1 and 25 ± 2, p<0.05, respectively). We found that 2dGal treated HUVECs showed decreased migration compared with nontreated HUVECs, towards RA SF (numbers of HUVEC migrated; 3 ± 1 and 34 ± 4, n=5 patients, p<0.05). Fractalkine/CX3CL1, CXCL16, or IL-8/CXCL8 in 2-d Gal treated HUVEC conditioned medium were decreased compared with in non-treated HUVEC conditioned medium but not MCP-1/CCL2, ENA-78/CXCL5, or VEGF.

Conclusion: These data indicate that glycoproteins are involved with RA, and play a role in angiogenesis in RA and suggest that targeting glycosylation especially fucosylation may provide a method by which to decrease inflammation and potentially treat other inflammatory diseases.

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THU0020 COMPARATIVE STUDY OF THE PHOSPHODIESTERASE TYPE 5 INHIBITOR SILDENAFIL AND THE PROSTACYCLIN ANALOGUE ILOPROST ON IP10 MODULATION IN SYSTEMIC SCLEROSIS

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Background: Systemic sclerosis (SSc) is an autoimmune disease ending in multiorgan fibrosis. Vascular damage, responsible for the vascular alteration and disruption, has been suggested to play a key role in disease maintenance and progression [1]. Recent data demonstrated that high serum levels of the interferon (IFN)-γ-induced protein 10 (IP-10) in SSc patients correlated with peripheral vascular injury and increased in association with nailfold capillaroscopic pattern worsening and digital ulcers presence [2, 3]. Between the vasoactive drugs used for SSc treatment, the prostacyclin analogue iloprost (I) and PDE-5 inhibitor sildenafil (S) seem to have high vasodilatory and immunomodulatory actions [4-6].

Objectives: To investigate and compare the ability of S and I to modulate: IP-10 circulating levels in SSc patients under different treatments; IP-10 release by human endothelial (Hfaec) cells subjected to Th1-related inflammatory stimuli.

Methods: Sera of 28 patients satisfying ACR/EULAR 2013 classification criteria for SSc were analyzed by ELISA. IFNγ+TNFα-induced activation of NFκB, STAT1, JNK, ERK1/2 and AKT in Hfaec after S or I was tested by Western blot.

Results: The treatment with S significantly reduced IP-10 serum levels vs. treatment with (DMARDS) and corticosteroids (CCs) (184.1±65.10 vs. 880.9±339.0 pg/ml and vs. 426.5±101.7, respectively, P<0.01); while no significant difference has been found vs. I (184.1±65.10 vs. 282.7±46.6 pg/ml). In Hfaec, S and I differently counteracted the IFN-γ/TNF-α-induced phosphorylation of JNKs (respectively 61.6±20.1% and 9.5±13.1% of phosphorylation vs. I+T-induced taken as 100%), STAT1 (respectively 49.2±15.8% and 93.4±1.2% of phosphorylation vs. I+T-induced taken as 100%), NFκB (respectively 72.6±8.3% and 92.2±3.3% of phosphorylation vs. I+T-induced taken as 100%), ERK1/2 (respectively 31.6±7.9% and 27.3±4.4% of phosphorylation vs. I+T-induced taken as 100%), and AKT (respectively 85.7±5.6% and 3.8±7.4% of phosphorylation vs. I+T-induced taken as 100%).

Conclusion: *In vivo* results show that S and I have more effect in targeting serum IP-10 in SSc patients than other therapeutic treatments. *In vitro* data show different inhibitory drug-induced effects onto paths underlying IP-10 production, depending on intracellular targets. S could be a potential pharmacological tool as effective as I to control IP-10 in blood or at endothelial cell level in SSc.

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